RESIDUES AND TRACE ELEMENTS

Validation of a Fast and Easy Method for the Determination of Residues from 229 Pesticides in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detection

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Validation experiments were conducted of a simple, fast, and inexpensive method for the determination of 229 pesticides fortified at 10–100 ng/g in lettuce and orange matrixes. The method is known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for pesticide residues in foods. The procedure involved the extraction of a 15 g sample with 15 mL acetonitrile, followed by a liquid–liquid partitioning step performed by adding 6 g anhydrous $MgSO_4$ plus 1.5 g NaCl. After centrifugation, the extract was decanted into a tube containing 300 mg primary secondary amine (PSA) sorbent plus 1.8 g anhydrous $MgSO_4$, which constituted a cleanup procedure called dispersive solid-phase extraction (dispersive SPE). After a second shaking and centrifugation step, the acetonitrile extract was transferred to autosampler vials for concurrent analysis by gas chromatography/mass spectrometry with an ion trap instrument and liquid chromatography/tandem mass spectrometry with a triple quadrupole instrument using electrospray ionization. Each analytical method was designed to analyze 144 pesticides, with 59 targeted by both instruments. Recoveries for all but 11 of the analytes in at least one of the matrixes were between 70–120% (90–110% for 206 pesticides), and repeatabilities typically <10% were achieved for a wide range of fortified pesticides, including methamidophos, spinosad, imidacloprid, and imazalil. Dispersive SPE with PSA retained carboxylic acids (e.g., daminozide), and <50% recoveries were obtained for asulam, pyridate, dicofol, thiram, and chlorothalonil. Many actual samples and proficiency test samples were analyzed by the method, and the results compared favorably with those from traditional methods.

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In 2003, Anastassiades et al. (1) introduced a new method of analysis to monitor produce for pesticide residues, which they termed the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method. The method utilizes acetonitrile (MeCN) for extraction (1 mL MeCN/1 g sample) using Vortex mixing followed by addition of 4 + 1 (w/w) anhydrous $MgSO_4$:NaCl (0.5 g salts per g sample) to induce partitioning of the MeCN extract from the water in the sample. After centrifugation, 1 mL of the extract is mixed with 25 mg primary secondary amine (PSA) sorbent + 150 mg anhydrous $MgSO_4$ in a simple approach that is termed dispersive solid-phase extraction (dispersive SPE) cleanup. The extract is centrifuged again and transferred to an autosampler vial for analysis by gas chromatography/mass spectrometry (GC/MS) and/or other technique.

The QuEChERS method has several advantages over traditional methods of pesticide residue analysis (2–6), as follows: (*1*) high recoveries (>85%) are achieved for a wide polarity and volatility range of pesticides, including notoriously difficult analytes such as methamidophos, omethoate, imazalil, thiabendazole, dichlorvos, and pyrethroids; (*2*) very accurate (true and precise) results are achieved because an internal standard (IS) is used to correct for commodity-to-commodity water content differences and volume fluctations; (*3*) high sample throughput of about 10–20 preweighed samples in 30–40 min can be achieved; (*4*) solvent usage and waste are very small, and no chlorinated solvents are used; (*5*) a single person can perform the method without much training or technical skill; (*6*) only a single reusable piece of labware (a 50 mL Teflon centrifuge tube) is used per sample, which is very easy to clean; (*7*) despite its speed and ease, the method is still quite rugged because extract cleanup is done to remove fatty acids and other organic

acids that are ubiquitous in foods; (*8*) very little bench space is needed, thus the method can be done in a small mobile laboratory if needed; (*9*) the MeCN is added by dispenser to an unbreakable vessel that is immediately sealed, thus solvent exposure to the worker is minimal (and no chlorinated solvent is used); (*10*) only $1 (US) of materials is used per 10 g sample, thus the method is very inexpensive; and (*11*) the only devices needed are a chopper, balance, and centrifuge (no blender, SPE manifold, or evaporation apparatus) to carry out the sample preparation method.

The main disadvantage of the QuEChERS method compared to other common methods is that the 1 g/mL final extract concentration is lower than the 2–5 g/mL concentrated extracts of most traditional methods. If matrix is not the limiting source of noise in the analysis, this leads to a higher limit of quantitation (LOQ) for the same injection volume in the QuEChERS method. Also, MeCN has a large vaporization expansion volume, which tends to further limit the GC injection volume. However, either solvent exchange/concentration into toluene or large volume injection (LVI) can be employed to potentially compensate for this difference so that adequately low LOQ can be achieved (7–10). In this study, we chose to utilize LVI for GC/MS analysis by injecting 5 L of the final MeCN extract onto a plug of Carbofrit contained in the liner of a programmable temperature vaporization (PTV) injector (7). The goal was to achieve an LOQ of at least 10 ng/g for the pesticide residues, which is needed to meet recent European Union (EU) legislation for baby foods (11).

In the previous paper (1), the QuEChERS method was demonstrated to be effective for 22 diverse GC-amenable pesticides in several fruit and vegetable commodities. The pesticides and commodities were carefully chosen to indicate that the method would likely work equally well for many other pesticides and commodities of interest, but this was not proven. Furthermore, liquid chromatography (LC) was not used in the initial study, thus the applicability of the QuEChERS approach to thermally labile and very polar pesticides was not evaluated. Only recently has LC tandem mass spectrometry (MS/MS) become viable for routine use to simultaneously monitor scores of pesticides at <10 ng/g levels in complicated matrix extracts (12–14). The combination of LC/MS/MS with GC/MS can currently provide the most effective and efficient means to both quantify and identify hundreds of pesticide analytes in a variety of matrixes.

The purpose of this study was to conduct a rather thorough validation of the QuEChERS sample preparation method for some 229 pesticides fortified from 10–100 ng/g in 2 representative commodities (lettuce and orange) using concurrent LVI/GC/MS and LC/MS/MS detection methods routinely used at the VWA-KvW (Food Inspection Service) laboratory in Amsterdam. Validation also entailed the analysis of many proficiency test samples and routine samples, and the results were compared to those obtained for the same samples when using traditional methods. In particular, the QuEChERS method was compared with the already very streamlined acetone-based method (15–21) in use since the early 1980s at the VWA-KvW laboratory in the Dutch enforcement and monitoring program.

Experimental

Apparatus

(**a**) *GC/MS instrument.*—The extracts were analyzed with a Varian (Walnut Creek, CA) Saturn 2000 instrument, in which the ion trap mass spectrometer was coupled to a Model 3800 gas chromatograph. The mass spectrometer was used in full-scan mode with electron impact (EI) ionization. The system was equipped with a Model 1079 PTV injector, electronic flow control, and a Model 8400 autosampler. The injection liner (single-gooseneck, 3.4 mm id) contained a plug of Carbofrit (Restek; Bellefonte, PA) to allow 5 L injection of the MeCN extracts. Saturn Workstation software was used for instrument control and data analysis.

(**b**) *LC/MS/MS instrument.*—The extracts were also analyzed with a Waters/Micromass (Manchester, UK) Quattro Ultima triple quadrupole instrument using electrospray ionization in the positive ion mode (ESI+). The LC instrument was a Waters Millenium Module Model 2695 equipped with a quaternary solvent delivery system, autosampler, and column heater. MassLynx software was used for instrument control and QuanLynx for data analysis.

(**c**) *Chopper and mixers.*—A 12 L volume Stephan (Hameln, Germany) UM12 cutting chopper and Polytron (Luzerne, Switzerland) PT 6000 homogenizer were used to comminute fruit and vegetable samples.

(**d**) *Centrifuge.*—For the 50 mL centrifuge tubes, a Sigma (Ostenrode am Harz, Germany) E3-1 centrifuge was utilized.

(**e**) *Liquid dispensers.*—An adjustable-volume solvent dispenser was used to conveniently provide 15 mL MeCN from the bottle to the samples. An adjustable repeating pipet was used to transfer the 0.36, 0.64, and 1 mL volumes to autosampler vials for analysis of the extracts. Gas-tight glass syringes with Teflon-tipped plungers were used to fortify samples, add the IS solution, and prepare calibration standards.

(**f**) *Analytical balance.*—A top-loading balance with digital display was used to weigh the chopped samples and powder reagents.

(**g**) *Vials and vessels.*—For both the extraction and dispersive-SPE cleanup steps, 50 mL fluorinated ethylene propylene (FEP) centrifuge tubes with ethylene–tetrafluoroethylene screw closures (Nalgene, Rochester, NY) were employed. Sealable 15 mL glass screw-cap vials were used to contain the 6 g anhydrous $MgSO_4$ + 1.5 g NaCl for the method. Standard 1.8 mL dark glass autosampler vials were used to contain the final extracts.

Reagents

(**a**) *MeCN, methanol (MeOH), and water.*—The organic solvents were of sufficient quality for pesticide residue analysis and were obtained from Labscan (Dublin, Ireland). Deionized water was used for preparing the LC mobile phase and as a reagent blank.

(**b**) *$MgSO_4$ and NaCl.*—Reagent grade anhydrous $MgSO_4$ in powder form and ACS grade NaCl were obtained from Merck (Darmstadt, Germany). The $MgSO_4$ was baked for 5 h at 500 C in a muffle furnace to remove phthalates.

(**c**) *Organic acids.*—Glacial acetic acid (HAc) and formic acid (both from Merck) were used to improve stability of base-sensitive pesticides in the final extracts and as an acid modifier of the LC mobile phase, respectively.

(**d**) *Pesticide standards.*—Pesticide reference standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Sigma-Aldrich/Fluka/Riedel-de-Haen (Zwijndrecht, The Netherlands). Stock solutions of 1.00 mg/mL were prepared in toluene and methanol for the pesticides included in the GC/MS and LC/MS/MS methods, respectively, and working standards containing 229 pesticides at 5 and 0.5 ng/ L were prepared in MeCN. To prepare the 5 g/mL mixture of 229 pesticides, the many nonvolatile pesticides were added first, and excess toluene and MeOH were periodically evaporated in the volumetric flask using a stream of N_2, then the relatively volatile pesticides were added before adding MeCN to the mark.

(**e**) *Internal standards.*—Triphenylphosphate (TPP) was obtained from Merck and ethoprophos from Dr. Ehrenstorfer. A solution of 2 ng/ L TPP in MeCN containing 2% HAc (v/v), which was added to the final extracts, was used as the quality control (QC) standard for the GC analytical step. A 5 ng/ L ethoprophos solution in MeCN was added to the sample to serve as the IS and QC standard for the entire method.

(**f**) *SPE sorbent.*—PSA sorbent (40 m particle size) was obtained from Varian (Harbor City, CA).

(**g**) *Fruit and vegetable samples.*—Blank samples of oranges and lettuce were purchased from a local organic produce store in Amsterdam. These were used in fortification experiments and as matrix blanks for matrix-matched calibration standards. The routine sample commodities analyzed consisted of cherry, nectarine, orange, apricot, pineapple, French bean, cucumber, eggplant, green cabbage, potato, avocado, lemon, clementine, grape, plum, and peach, which were obtained from the Central Enforcement Team for pesticide analysis in the Dutch VWA-KvW laboratory. The proficiency test samples analyzed consisted of spinach, sweet pepper, orange, apple, lettuce, carrot, melon, and strawberry, which were obtained from Food Analysis Performance Assessment Scheme (FAPAS®; York, UK) and the European Commission's EU Proficiency Test on Pesticide Residues in Fruit and Vegetables organized by Sweden's National Food Administration (NFA; Uppsala, Sweden).

Preliminary Steps and Sample Comminution

Many vials containing 6.00 ± 0.15 g anhydrous MgSO4 + 1.50 ± 0.15 g NaCl were prepared in advance and stored capped at room temperature until needed in experiments. The necessary number of 50 mL FEP centrifuge tubes containing 0.30 ± 0.02 g PSA + 1.8 ± 0.2 g anhydrous $MgSO_4$ were prepared just prior to experiments.

For sample comminution, a large chopper was used to comminute the 1–5 kg representative laboratory sample. The sample was blended until it gave a consistent texture. Then, ca 200 g was transferred to a screw-top container, and the subsample was mixed with the probe blender/homogenizer until it was homogeneous. A second representative subsample (test portion) of 15 g was taken for extraction immediately, and the container was then sealed and stored in the freezer in case reanalysis was necessary. The advantages of this approach are that the 15 g portion is highly representative of the initial sample, the sample is well-comminuted to improve shaking-based extraction, less time is spent on the overall homogenization process than trying to provide equivalent homogenization of the large initial sample with just the chopper, and a frozen subsample is available for reanalysis if needed.

QuEChERS Extraction and Cleanup Procedure

In validation experiments, lettuce and orange commodities were used as representative matrixes. In each case, 6 replicates each at 3 levels (10, 50, and 100 ng/g in lettuce and 10, 25, and 100 ng/g in orange) were fortified into the samples. The rapid procedure described below was followed for extraction and cleanup: (*1*) Weigh 15.00 ± 0.05 g of thoroughly comminuted sample into a 50 mL FEP centrifuge tube (use 13 mL deionized water for a reagent blank); a 15 g blank was prepared as the matrix blank and for matrix-matched calibration standards. (*2*) Fortify the sample with 300 L of the 0.5 ng/ L spiking mixture, or 75, 150, and 300 L of the 5 ng/ L mixture, to yield 10 or 25, 50, and 100 ng/g concentrations, respectively. (*3*) Add 15 mL MeCN into each tube using the solvent dispenser and 300 L of the 5 ng/ L ethoprophos solution in MeCN to all samples except blanks (this gives a 100 ng/g equivalent concentration). (*4*) Cap the tubes well and shake vigorously by hand for 45 s (3–5 tubes can be placed in each hand). (*5*) Uncap the tubes and add the 6 g anhydrous $MgSO_4$ and 1.5 g NaCl (do not get the powders in the threads or rims of the tubes). (*6*) Repeat step 4, ensuring that the solvent interacts well with the entire sample and that crystalline agglomerates are broken up sufficiently during shaking. (*7*) Centrifuge the tubes at 3000 rpm for 1 min. (*8*) Decant the extracts (upper layer) into the dispersive-SPE tubes containing 0.3 g PSA + 1.8 g anhydrous $MgSO_4$. (*9*) Cap the tubes well and shake them by hand for 20 s. (*10*) Repeat step 7.

Routine Dutch Acetone Method

For comparison purposes, the method developed in 1983 and continually applied since then in the Dutch monitoring and enforcement program was also used to analyze proficiency test samples and a batch of routine samples. This method has been described elsewhere (15–21) but, in brief, it is a miniatiurized and streamlined version of the Luke method (2). In the Dutch approach, 15 g sample (or 7.5 g for the alternative acetone + Na_2SO_4 extraction method for polar pesticides) is placed in a 250 mL Teflon centrifuge tube, which is blended using a probe blender for 20 s with 30 mL

Table 1. Parameters for the GC/MS analysis of pesticides in the lettuce experiment (minor differences occurred in t_R and chosen quantitation ions for oranges). Underlined analytes were detected by LC/MS/MS, also

#	t_R, min	Pesticide	Quantitation ion(s)
1	6.102	Dichlorvos	109+185
2	6.119	Methamidophos	94+141
3	7.028	Diphenyl	154
4	7.199	Mevinphos	127
5	7.304	Acephate	136
6	7.360	Etridiazole	211
7	7.407	Propham	179
8	7.596	Phthalimide	147
9	7.719	Tetrahydrophthalimide	79
10	7.856	*o*-Phenylphenol	169
11	8.119	Heptenophos	215
12	8.343	Omethoate	156
13	8.396	Propoxur	110
14	8.549	Ethoprophos (IS)	158+159
15	8.590	Diphenylamine	169
16	8.757	Chlorpropham	213
17	8.873	DMSA	92
18	8.918	Cadusafos	159
19	8.979	Monocrotophos	127
20	9.056	Pencycuron	180
21	9.094	Phosmet-oxon	160
22	9.253	Hexachlorobenzene	284
23	9.437	Dimethoate	93+125
24	9.474	Fenpyroximate	213
25	9.470	Carbofuran	164
26	9.500	Dicloran	176
27	9.782	DMST	106
28	9.789	Diazinon	304
29	9.805	Lindane	181
30	9.848	Propyzamide	173
31	10.018	Pyrimethanil	198
32	10.165	Chlorothalonil	266
33	10.375	Pirimicarb	166
34	10.488	Furmecyclox	123
35	10.651	Pirimicarb-desmethyl	152
36	10.804	Spiroxamine I[a]	100
37	10.882	Chlorpyrifos-methyl	286
38	10.941	Vinclozolin	212+198+214+200
39	11.050	Tolclofos-methyl	265
40	11.077	Parathion-methyl	263
41	11.190	Metalaxyl	206
42	11.224	Prometryn	184+241+242
43	11.286	Carbaryl	144
44	11.318	Fenpropidin	98
45	11.386	Spiroxamine II[a]	100
46	11.452	Pirimifos-methyl	290+276+305
47	11.628	Fenithrothion	260+277
48	11.687	Methiocarb	168+153
49	11.741	Malathion	173
50	11.803	Dichlofluanid	123+224+167
51	11.906	Chlorpyrifos	314+316
52	11.953	Diethofencarb	267+225
53	12.054	Aldrin	263+293
54	12.074	Fenthion	278
55	12.092	Chlorthaldimethyl	301
56	12.159	Parathion	291
57	12.214	Triadimefon	208
58	12.237	Tetraconazole	336
59	12.372	Dichlorobenzophenone	250+139
60	12.609	Fenpropimorph	128
61	12.746	-Chlorfenvinphos	267+269+323
62	12.856	Cyprodinil	224
63	12.973	Penconazole	248
64	12.983	Chlozolinate	259+261+186+188+190
65	13.040	-Chlorfenvinphos	267+269+323
66	13.040	Tolylfluanid	323+325+267+269
67	13.102	Mecarbam	131
68	13.232	Quinalphos	146
69	13.313	Furalaxyl	242
70	13.306	Mephosfolan	227+269
71	13.378	Triflumizole	278
72	13.342	Triadimenol	112
73	13.380	Procymidone	283+285
74	13.454	Captan	79+149
75	13.587	Folpet	260+262
76	13.716	Methidathion	85+145
77	13.771	Pyrifenox	262+264
78	14.066	Picoxystrobin	335
79	14.168	Mepanipyrim	222
80	14.362	Prothiofos	309
81	14.362	-Endosulfan	241+239
82	14.355	Hexaconazole	309
83	14.375	Flutolanil	173+281
84	14.522	Profenofos	339+337
85	14.847	Buprofezin	175+105
86	14.863	Myclobutanil	179
87	14.897	Bupirimate	208+273
88	14.901	Flusilazole	233
89	14.917	Fludioxonil	248

Table 1. (*continued*)

#	t_R, min	Pesticide	Quantitation ion(s)
90	14.956	Kresoxim-methyl	131
91	15.468	Cyproconazole	222
92	15.802	Diniconazole	268+270
93	15.846	Ethion	231
94	15.833	Fenthion-sulfoxide	279
95	15.876	-Endosulfan	241+239+195+243+197
96	16.084	Oxadixyl	163+132
97	16.441	Mepronil	119
98	16.514	Triazophos	257
99	16.700	Ofurace	232
100	16.844	Trifloxystrobin	116
101	16.958	Quinoxyfen	237+272+307
102	17.085	Endosulfan sulfate	387+389+385+270
103	17.073	Propiconazole	259+261
104	17.139	Fenhexamid	177+266
105	17.646	Propargite	335+350+135
106	17.560	Tebuconazole	250
107	17.696	Diflufenican	266+394
108	17.714	Piperonyl butoxide	176
109	17.742	TPP (IS)	325+326
110	18.095	Epoxiconazole	192
111	18.432	Pyridaphenthion	340
112	18.473	Iprodione	314+316
113	18.555	Bifenthrin	181+165+167
114	18.674	Bromopropylate	341+339+343
115	18.726	Phosmet	160
116	18.743	EPN	169+141+157
117	18.783	Fenpiclonil	236+238
118	18.878	Fenoxycarb	88+116
119	18.949	Fenpropathrin	181+265
120	19.075	Dicofol	139+251
121	19.100	Tebufenpyrad	318+276
122	19.226	Fenazaquin	145
123	19.354	Bromuconazole	295+293
124	19.670	Tetradifon	358+356+354+229+231
125	19.811	Phosalone	367+182
126	20.064	Azinphos-methyl	160
127	20.070	Cyhalothrin	181+197
128	20.113	Pyriproxyfen	136
129	20.680	Acrinathrin	289+181
130	20.680	-Cyhalothrin	197+181
131	20.791	Pyrazophos	221
132	20.841	Fenarimol	139
133	21.789	Bitertanol	170
134	21.862	*cis*-Permethrin	183
135	22.079	Pyridaben	309+147
136	22.102	*trans*-Permethrin	183
137	22.268	Fluquinconazole	340
138	23.583	Cypermethrin	181+163+165
139	23.785	Flucythrinate I[a]	199+157+225
140	24.030	Etofenprox	163
141	24.173	Flucythrinate II[a]	199+157+225
142	25.122	Fenvalerate	225
143	25.337	Fluvalinate	250
144	25.528	Esfenvalerate	225
145	26.290	Difenoconazole	323+325+265+267
146	26.585	Deltamethrin	253
147	27.092	Azoxystrobin	344
148	27.369	Famoxadone	330+224+196

[a] Spiroxamine and flucythrinate standards were mixtures of isomers resulting in 2 separate GC peaks (indicated as I and II). Other isomers of pesticides indicated individually in the table were added to the mixture as pure individual neat standards.

acetone. Then, 30 mL dichloromethane + 30 mL petroleum ether (followed by 7.5 g Na_2SO_4 for the alternative extraction method) are added, and the sample extract is blended again (partitioning step) for only 20 s. After centrifugation, a 15 mL portion of the upper layer is placed in a calibrated test tube with some boiling chips, and batchwise evaporation of the extracts is conducted in a water bath in a hood, with a bath temperature starting at 40 C and continuing to 62 C until near dryness. The last part of solvent is allowed to evaporate by placing the tube rack next to the water bath in the hood for gentle evaporation. For GC/MS (and optionally for other GC detection systems), the extract is brought up to 3 mL in iso-octane–toluene (9 + 1), yielding a 0.9 g sample equivalent/mL extract (after applying an average volume correction). For LC/MS/MS, a 2 mL portion (0.36 g equivalent extract) is evaporated until dryness and brought up to 1 mL with MeOH.

Preparation of Matrix-Matched Calibration Standards

The extracts from Step 10 above were transferred to separate autosampler vials for GC/MS and LC/MS/MS analyses. First, 1 mL extracts were transferred to the appropriately labeled autosampler vials for GC/MS. Then, 50 L of 2 ng/ L TPP in MeCN containing 2% HAc (v/v) was added to each extract including blanks. This provides a ca 100 ng/g equivalent concentration of TPP to extracts and standards alike, and the 0.1% final HAc concentration helps stabilize certain pesticides in the MeCN extract (22). In the case of fortification experiments, calibration standards were prepared in matrix-matched solutions (standards added to

blank extracts) as follows: 20 or 50 L of the 0.5 ng/ L pesticide mixture in MeCN was added to 1 mL blank extracts for the 10 and 25 ng/g equivalent standards, respectively; 10 and 20 L of the 5 ng/ L pesticide mixture was added to 1 mL blank extracts for the 50 and 100 ng/g equivalent standards, respectively. Calibration standards in blank orange and lettuce extracts ranging from 10–2500 ng/g equivalents were used for the analysis of the real samples and proficiency test samples. Ethoprophos (20 L) was added as the IS to the calibration standards in each case, and an appropriate amount of MeCN was added to all extracts to make a consistent final volume of 1.12 mL prior to transfer of an aliquot for LC/MS/MS analysis. After the contents of all vials were mixed well, 0.36 mL of each final solution was transferred to the appropriately labeled LC/MS/MS autosampler vial. Then, 0.64 mL MeOH was added, the vials were capped and shaken, and they were placed in the autosampler tray for sequential analysis. Calibration standards were also prepared in solvents (MeCN for GC/MS and MeCN–MeOH for LC/MS/MS) for comparison purposes.

GC/MS and LC/MS/MS Analyses

GC analysis was conducted on a CP-Sil 8-ms (Varian, Middelburg, The Netherlands) capillary column (30 m, 0.25 mm id, 0.25 m film thickness) with the following conditions: He constant flow, 1.3 mL/min; initial inlet temperature, 80 C ramped to 280 C at 200 C/min after a 30 s delay; injection volume, 5 L (LVI) onto a Carbofrit plug in the liner with an open purge valve (30:1 split ratio) for 24 s, closed until 3.5 min, and open again (30:1) until the end of the run; oven temperature program, 75 C for 3 min, then 25 C/min ramp to 180 C followed by a 5 C/min ramp to 300 C and held for 3 min (total run time: 34.2 min). The MS instrument transfer line temperature was 240 C, with 230 C ion trap and 120 C manifold temperatures. Full-scan (60–550 *m/z*) EI (auto mode) with 10 A filament current was used for MS analysis from 5–31 min, which gave 2.7 scans/s. Target automatic gain control was 15 000, and the multiplier voltage was 1450 V. Table 1 gives the particular conditions for the pesticides analyzed by GC/MS.

For LC/MS/MS, the injection volume was 5 L onto a 15 cm long, 3 mm id, 5 m particle size Alltima C_{18} column (Alltech, Deerfield, IL). Flow rate was 0.3 mL/min, and a gradient program was used consisting of MeOH–water (25 + 75, v/v) containing 5mM formic acid ramped linearly over the course of 15 min to MeOH–water (95 + 5, v/v) containing 5mM formic acid, which was held at these conditions for another 15 min (total run time: 30 min). Typical MS instrument source conditions in ESI+ mode were as follows: capillary voltage, 2.0 kV; sample cone voltage, 35 V; source temperature, 100 C; and drying gas temperature, 350 C. Nebulizing gas and drying gas (N_2) flow rates were 100 and 500 L/h, respectively. MS/MS conditions were optimized for each pesticide by infusion and will be reported elsewhere (23).

Results and Discussion

Certain modifications in the initial QuEChERS method (1) were made to accommodate the operations, devices, and instrumentation at VWA-KvW, where this study was conducted. First of all, the VWA-KvW multiresidue method calls for a 15 g sample (15–21), and we chose to scale up the QuEChERS protocol from a 10 to a 15 g sample to maintain continuity and permit better comparison of results. The use of either sample size should provide equivalent results if proper sample homogenization procedures are followed with appropriate devices (24–26), but the 15 g sample plus extraction solvent and salts could still be contained satisfactorily within the 50 mL FEP centrifuge tubes used for extraction, so it was still convenient to use this amount. However, 15 g was deemed the maximum sample size of fruits and vegetables that could be used for the tubes.

A second change in the original QuEChERS procedure was related to the first in that the 15 g samples + 15 mL MeCN + 6 g anhydrous $MgSO_4$ + 1.5 g NaCl did not adequately mix using just a Vortex mixer as described in the original report (1). We found that shaking the tubes by hand (mainly using a motion from the shoulders and elbows more so than the wrist) provided better mixing and, in fact, this modification increased sample throughput because 3–5 tubes could be held in each hand and shaken together whereas only 1 tube could be mixed at a time using a standard Vortex mixer. Also, the agglomerates that occurred when the $MgSO_4$ hydrated with water in the sample were better broken apart by hand-shaking, which permits inversion of the tubes and better control of the mixing process. Moreover, the strong vibrations of the Vortex mixer were potentially damaging to the hand in long-term, routine operations, and shaking eliminated this concern. A strong mechanical shaker could be substituted for manual shaking, but capital expense and space needs would increase while sample throughput would decrease due to the extra time needed to place and remove the tubes from the shaker. The manual exertion to shake the tubes, however, may vary from person to person. In any event, the sample homogenization process is more important than the shaking process to provide the extraction solvent with good access to the sample (1). As described in the *Experimental* section, a 2-stage sample comminution procedure was followed in this study (and all analyses conducted in the VWA-KvW laboratory) that provided well-homogenized 15 g test portions ready for extraction by shaking.

The third modification to the initial QuEChERS method was the decanting of the entire extract after centrifugation to a second 50 mL FEP centrifuge tube for dispersive SPE with 300 mg PSA sorbent + 1.8 g anhydrous $MgSO_4$. Previously, the method called for cleanup of 1 mL extract with 25 mg PSA + 150 mg anhydrous $MgSO_4$. The option to take a small aliquot had advantages in terms of reducing cost, but we chose to make the change for the following reasons: (*1*) the 0.5 mL from the original method was a bit too small for concurrent GC/MS and LC/MS/MS analyses in separate autosampler vials; (*2*) the 15 g sample in the tube and swinging bucket

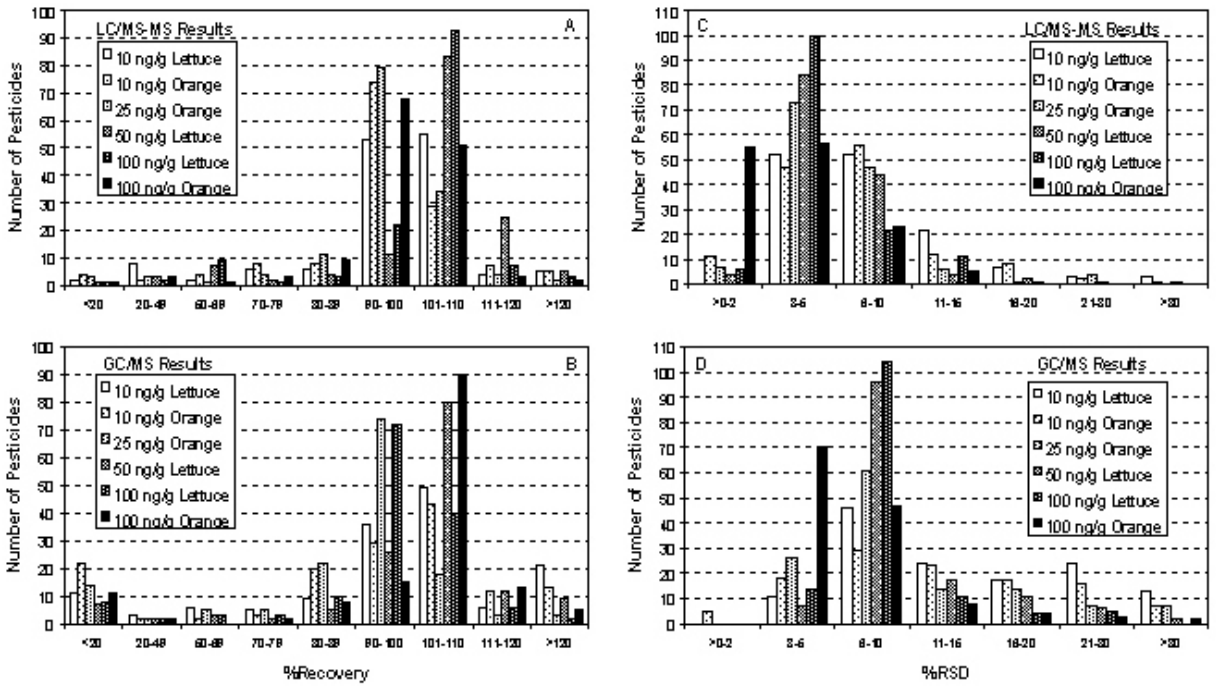

Figure 1. Pesticide recovery profiles from the validation experiments using LC/MS/MS and GC/MS at different levels in fortified lettuce and orange samples: (A) and (B) recoveries from LC/MS/MS and GC/MS, respectively, and (C) and (D) repeatabilities (*n* = 6) from LC/MS/MS and GC/MS, respectively.

centrifuge formed a very solid plug that made decanting of the extract very simple and quick (no pipetting needed); (*3*) the entire extract underwent dispersive-SPE cleanup and was available for reanalysis or other purposes (e.g., possible matrix blanks); and (*4*) the same centrifuge, rotor, and tube holders were used for centrifugation after both the extraction and cleanup steps (a minicentrifuge or different holders would have been needed if minicentrifuge tubes had been used).

Despite the small differences in this protocol, the QuEChERS method provided the same type of extract and advantages as listed in the Introduction and previously (1). In this study, the final extracts were analyzed by both GC/MS and LC/MS/MS with 5 L injections (for LC/MS/MS, 0.36 mL MeCN extract was diluted with 0.64 mL MeOH prior to analysis). Unlike previously (1, 27), analyte protectants were not used in GC/MS due to untested factors in their combination with Carbofrit and full-scan MS on an ion trap instrument. Therefore, matrix matching was employed in calibration (pesticides were added to matrix blank extracts to serve as calibration standards). For comparison, calibration standards in solvent were also analyzed to assess the effect of the matrix on the signals. No matrix effects were observed in ESI LC/MS/MS in the case of lettuce, but ion suppression occurred in a small region (18–20 min) in the chromatograms of orange extracts. Further investigations on analyte protectants in GC/MS have been conducted, and they show promise to improve the LOQ, peak shapes, repeatability, ruggedness, and analyte identification, and to eliminate the need for matrix-matched standards in GC/MS (28). Unfortunately, they are not designed to overcome ion

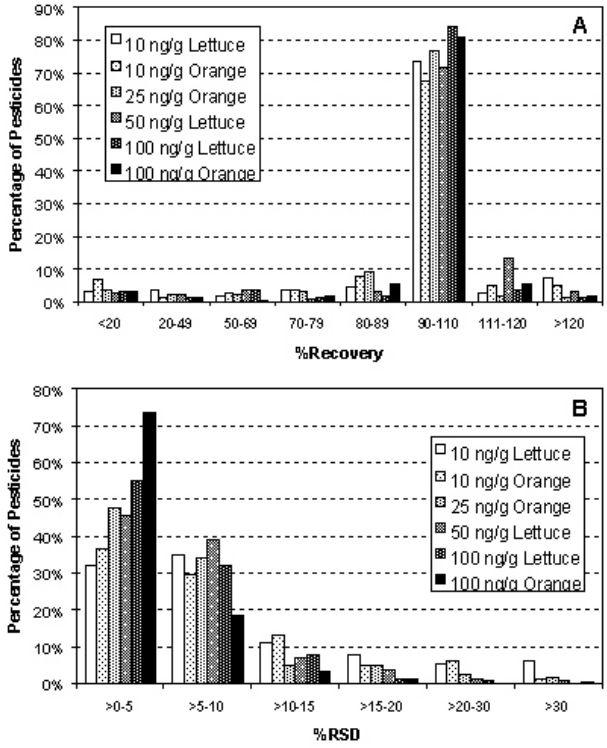

Figure 2. Overall recovery (A) and repeatability (B) profiles for the pesticides in orange and lettuce at different fortification levels using either LC/MS/MS or GC/MS for analysis of QuEChERS extracts.

suppression effects that can occur in LC/MS techniques. Other ionization modes or alternative techniques were not tested in this study to address the issue of ion suppression.

Comparison of LC/MS/MS and LVI/GC/MS

Figures 1 and 2 give the overall recovery and repeatability profiles of the results using LC/MS/MS and/or GC/MS for analysis of the different fortification levels in lettuce and orange. In all, 229 pesticides (including metabolites and some isomers) were fortified into the samples, 144 of which were analyzed by LC/MS/MS and another 144 by GC/MS with 59 overlapping pesticides detected on both instruments (as shown in Tables 1 and 2). Figure 1A and B exhibit the pesticide recoveries by LC/MS/MS and GC/MS, respectively, and Figure 1C and D show the repeatabilities of the results in terms of relative standard deviation (RSD) in the same manner.

As Figure 1 indicates, the LC/MS/MS analytical method outperformed the LVI/GC/MS (full-scan) method in the experiments. This is mainly caused by the better intrinsic instrument sensitivity and selectivity of the LC/MS/MS triple-quadrupole system used in the MS/MS mode compared with the GC/MS ion trap system used in the full-scan mode. The multireaction monitoring feature of the LC/MS/MS system, and much more user-friendly software, permitted much easier and faster peak identification, integration, and data processing operations than the GC/MS approach. Indeed, no outliers occurred in LC/MS/MS, but the GC/MS results contained many outliers that were eliminated in the final results. LC generally provides better reproducibility in the injection process, which is another reason for the better consistency in the results by LC/MS/MS. Some pesticides (11 in lettuce and 22 in orange) could not be detected at the 10 ng/g level by the GC/MS method, whereas LC/MS/MS had no trouble in detecting the 10 ng/g spikes for nearly all analytes in either matrix, despite that 66% less equivalent sample was introduced for LC than for GC. LC also avoids degradation problems with thermolabile pesticides, such as tolylfluanid, dichlofluanid, and certain carbamates. In fact, LC/MS/MS gave more reliable and/or sensitive results for all doubly analyzed pesticides except fenarimol, fenthion, and tebufenpyrad. Many pesticides, including these, typically gave the same recoveries with either instrument, but LC/MS/MS nearly always gave slightly less variability. Among the 59 overlapping pesticides, 21 gave the same quality of results by either analytical method, but LC/MS/MS clearly determined many important pesticides better, which most significantly included acephate, methamidophos, omethoate, monocrotophos, dimethoate, dichlorvos, fenthion sulfoxide, azinphos-methyl, pyridaphenthion, kresoxim-methyl, dichlofluanid, tolylfluanid, azoxystrobin, carbaryl, epiconazole, cyproconazole, bromuconazole, triflumizole, fenpropimorph, oxadixyl, pirimicarb desmethyl, furmecyclox, pyridaben, and mephosfolan.

The main conclusion from the comparison of the LC/MS/MS results with those from LVI/GC/MS is that LC/MS/MS is the preferred approach for those pesticides that are compatible in both analytical systems (at least for the instruments, techniques, methods, analytes, matrixes, and purpose investigated). The LC/MS/MS instrument employed in this study was much more expensive (ca $230 000) than the GC/MS instrument (ca $75 000), thus one would expect that an instrument of this caliber would provide such exceptional results. Ultimately, the LC/MS/MS results were selected in the final analysis for 140 analytes, and GC/MS for the remaining 89.

The GC/MS instrument was also capable of tandem MS (MS^n) analysis, but this feature was not used because it would be very difficult to devise conditions for so many pesticide analytes in a single GC run of reasonable time length (10). However, additional analytes could be moved to the LC/MS/MS method in the future to possibly improve their results. LC provides ca 10-fold wider peaks, thus many more coeluting analytes can be accurately monitored in an MS/MS segment. However, full-scan GC/MS has some advantages in often providing more information for pesticide identification (more ions and comparison with spectral libraries), including nontargeted chemicals in the sample. Both MS/MS and selected ion monitoring (SIM) only permit targeted analysis of a limited number of analytes in a chromatogram. A more detailed discussion of these issues and options is presented elsewhere (29, 30).

Validation Results

Figure 2 presents the combined recovery profiles using LC/MS/MS or GC/MS results for the 229 tested pesticides at each spiking level in both matrixes. Figure 2A demonstrates how the QuEChERS method gave 90–110% recoveries for 70–80% of the analytes, even at 10 ng/g in both matrixes. Most of the remaining pesticides still met typical validation requirements to achieve 70–120% recoveries, and only a small number did not meet these criteria, as discussed in the next section. In terms of repeatability, the vast majority of pesticides gave <10% RSD with $n = 6$ at each spiking level. Again, only a small number of pesticides gave >15% RSD, most of which occurred at the 10 ng/g level using GC/MS analysis. Differences due to analyte concentration were minimal in terms of recovery for nearly all analytes and, as usual, RSD increased somewhat as concentration decreased. A few pesticides were also affected at the 10 ng/g level in either orange or lettuce on both instruments. Small differences between matrixes can be discerned in Figures 1 and 2 and, interestingly, oranges gave slightly more reproducible results than lettuce, even though orange is generally conceived to be a more complicated matrix (mainly due to the peel). This may be related to the greater stability of pesticides, in general, in an acidic matrix (22).

The IS, ethoprophos, was verified to yield 100% recovery in the method and its use did not significantly affect recoveries, but it provided better repeatability and reproducibility in the GC/MS results. The analytical QC spike of TPP, which was added to the final extract prior to analysis, also gave very consistent results with an overall equivalent of 97 ± 5 "%recovery" versus ethoprophos IS in the GC/MS

Table 2. Parameters for the LC/MS/MS analysis of pesticides

#	t_R, min	Pesticide	MS/MS transition	
1	3.28	Daminozide	161	143
2	4.17	Methamidophos	142	94
3	4.64	Acephate	184	143
4	5.25	Butocarboxim sulfoxide	229	92
5	5.25	Omethoate	214	155
6	5.29	Pymetrozine	218	105
7	5.33	Oxamyl-oxime	163	72
8	5.76	Aldicarb sulfoxide	229	109
9	5.85	Methomyl-oxime	106	58
10	6.18	Asulam	231	156
11	6.25	Butocarboxim sulfone	245	130
12	6.54	Aldicarb sulfone	245	109
13	6.76[a]	Vamidothion sulfoxide	304	201
14	6.82	Oxamyl	237	72
15	7.59	Oxydemeton-methyl	247	169
16	7.93	Vamidothion sulfone	320	178
17	8.05	Demeton-S-methyl sulfone	263	169
18	8.13	Methomyl	163	106
19	8.33	Carbendazim	192	160
20	8.59	Thiamethoxam	292	211
21	8.79	Monocrotophos	224	127
22	9.36	Dicrotophos	238	112
23	9.58	Ethiofencarb sulfone	258	107
24	9.87	Thiofanox sulfoxide	257	200
25	9.90	Pirimicarb desmethyl	225	168
26	9.91	Ethiofencarb sulfoxide	242	107
27	10.25	Thiabendazole	202	175
28	10.27	Imidacloprid	256	209
29	10.43	Thiofanox sulfone	273	216
30	10.57	Methiocarb sulfoxide	242	122
31	10.81	Vamidothion	288	146
32	10.94	Carbofuran, 3-hydroxy	255	163
33	11.18	Trichlorphon	257	221
34	11.32	Dimethoate	230	171
35	11.41	Acetamiprid	223	126
36	11.53	Methiocarb sulfone	258	122
37	12.11	Cymoxanil	199	128
38	12.44	Thiacloprid	253	126
39	12.62	Florasulam	360	129
40	12.85	Ethirimol	210	140
41	13.09	Butocarboxim	213	75
42	13.23	Thiometon sulfoxide	263	185
43	13.26	DMSA	201	92
44	13.29	Aldicarb	213	116
45	13.30	Tricyclazole	190	136
46	13.36	Metoxuron	229	72
47	13.38	Oxadixyl	279	219
48	13.40	Thiometon sulfone	279	143
49	14.14	Azamethiphos	325	183
50	14.14	Pirimicarb	239	182
51	14.30	Mephosfolan	270	196
52	14.35	Thiophanate-methyl	343	151
53	14.47	Demeton-O-sulfoxide	275	141
54	14.47	Thiram	241	88
55	14.54	Propoxur	210	111
56	14.58	Imazalil	297	159
57	14.65	Carbofuran	222	165
58	14.72	Dichlorvos	221	127
59	14.89	DMST	215	106
60	14.98	Demeton-S-methyl	253	89
61	15.02	Fenthion sulfoxide	295	280
62	15.35	Dodemorph	282	116
63	15.36	Carbaryl	202	145
64	15.68	Ethiofencarb	226	107
65	15.74	Fosthiazate	284	104
66	15.74	Thiodicarb	355	88
67	15.90	Thiofanox	241	184
68	15.93	Monolinuron	215	126
69	16.07	Fenpropimorph	304	147
70	16.24	Thiometon	247	89
71	16.36	Spiroxamine I[b]	298	144
72	16.45	Metobromuron	259	170
73	16.56	Spiroxamine II[b]	298	144
74	16.59	Desmedipham	318	182
75	16.83	Phenmedipham	318	168
76	16.97	Azaconazole	300	159
77	17.03	Diuron	233	72
78	17.06	Azoxystrobin	404	372
79	17.25	Azinphos-methyl	340	132
80	17.31	Phosmet	318	160
81	17.35	Demeton	259	89
82	17.41	Diethofencarb	268	226
83	17.49[a]	Dimethomorph	388	301
84	17.50	Nuarimol	315	252
85	17.90	Methiocarb	226	169

Table 2. (*continued*)

#	t_R, min	Pesticide	MS/MS transition	
86	17.93	Linuron	249	160
87	17.93	Paclobutrazol	294	70
88	18.00	Tridemorph	298	116
89	18.04	Pyrimethanil	200	107
90	18.18[a]	Cyproconazole	292	70
91	18.18	Myclobutanil	289	70
92	18.18	Triadimefon	294	197
93	18.21	Isoprothiolane	291	189
94	18.21	Pyridaphenthion	341	189
95	18.25	Chlorbromuron	295	206
96	18.41	Spinosad A[b]	733	142
97	18.42	Triadimenol	296	70
98	18.46	Iprovalicarb	321	119
99	18.50[a]	Pyrifenox	295	93
100	18.50	Tetraconazole	372	159
101	18.50	Dichlofluanid	333	123
102	18.50	Fenhexamid	302	97
103	18.53	Bromuconazole	378	159
104	18.53	Flufenacet	364	152
105	18.74	Fenarimol	331	268
106	18.80	Bupirimate	317	166
107	18.83	Fenbuconazole	337	125
108	18.87	Epoxiconazole	330	121
109	18.90	Spinosad D[b]	747	142
110	18.94	Picoxystrobin	368	145
111	18.97	Etaconazole	328	159
112	18.97	Flusilazole	316	165
113	18.99	Tebufenozide	353	133
114	19.01	Fenamiphos	304	217
115	19.15	Fenoxycarb	302	116
116	19.34	Tolylfluanid	347	137
117	19.36	Diclobutrazole	328	70
118	19.45	Kresoxim-methyl	314	267
119	19.54	Tebuconazole	308	70
120	19.68	Penconazole	284	159
121	19.78	Propiconazole	342	159
122	19.82	Furmecyclox	252	170
123	19.82	Fenthion	279	169
124	19.89	Bitertanol	338	269
125	19.89	Cyprodinil	226	93
126	19.93	Isoxathion	314	105
127	19.96	Hexaconazole	314	70
128	19.96	Metconazole	320	70
129	19.96	Prochloraz	376	308
130	20.07	Pencycuron	329	125
131	20.15	Trifloxystrobin	409	186
132	20.27	Difenoconazole	406	251
133	20.37	Diniconazole	326	70
134	20.49	Clofentezine	303	138
135	20.54	Triflumizole	346	278
136	20.92	Furathiocarb	383	195
137	21.02	Profenofos	375	305
138	21.08	Buprofezin	306	201
139	21.08	Tebufenpyrad	334	145
140	21.10	Cycloxydim	326	280
141	21.41	Sethoxydim	328	178
142	21.78	Hexythiazox	353	168
143	22.86	Fenpyroximate	422	366
144	23.33	Pyridaben	365	309
145	24.32	Pyridate	379	207
146	25.63	Etofenprox	394	177

[a] 1st (major) of 2 peaks, used for quantitation.

[b] In the cases of spiroxamine I and II and spinosad A and D, both peaks were used for quantitation. Their standards were mixtures of isomers resulting in 2 separate LC peaks.

analysis of both matrixes. In LC/MS/MS, only external standard calibration was applied.

The overall pesticide recoveries and RSD were compiled for each matrix and for both matrixes (typically, $n = 18$ and $n = 36$, respectively), and Table 3 lists each pesticide in the given categories. The use of capitalization, italics, bold font, and underlining (or not), as described in the table caption, provides information about each listed pesticide. Depending on the matrix, the vast majority, 208 of the 229 pesticides, gave overall recoveries of 90–110%. The recoveries of 6 other pesticides (acephate, methamidophos, cycloxydim, fenhexamid, pymetrozine, and fenvalerate) fell between 80–89%, and 3 others (cypermethrin, florasulam, and dichlofluanid) consistently gave 70–79% overall recovery in at least one of the matrixes. A significant high bias was not encountered with the method, and the only analytes to yield >110% average recoveries in both matrixes were degradation products of dichlofluanid and tolylfluanid, N,N-dimethyl-N-phenylsulphamide (DMSA) and dimethylaminosulfotoluidide (DMST), respectively. Their recoveries were high due to the partial degradation of the parent pesticides.

Table 3. List of pesticides grouped by their overall recoveries, compiled from 3 spiking levels each in orange and lettuce matrixes. Pesticides with results using GC/MS data are capitalized, and those from LC/MS/MS are not capitalized (those analyzed by both instruments are underlined). Pesticides in italics indicate results from lettuce only, and those in bold denote orange results. Those pesticides marked with an asterisk indicate that a 10 ng/g result in one of the matrixes was an outlier due to background interferences or low signal/noise.

Pesticides with >110% recovery: *azamethiphos*, *carbendazim*, *Cyhalothrin*, dmsa, dmst, **Phthalimide**, *Tetradifon*, **Tetrahydrophthalimide**

Pesticides with 90–110% recovery (and ≤10% RSD): acetamiprid, Acrinathrin*, aldicarb, aldicarb sulfone, aldicarb sulfoxide, Aldrin, azaconazole, **azamethiphos**, azinphos-methyl, azoxystrobin, Bifenthrin, bitertanol, Bromopropylate, bromuconazole, Bupirimate, buprofezin, butocarboxim, butocarboxim sulfone, butocarboxim sulfoxide, Cadusafos, carbaryl, **carbendazim**, carbofuran, 3-hydroxy-carbofuran*, chlorbromuron, -Chlorfenvinphos, -Chlorfenvinphos, Chlorpyrifos, Chlorpyrifos-methyl, Chlorthaldimethyl, Chlozolinate, clofentezine, **Cyhalothrin**, *cymoxanil*, cyproconazole, cyprodinil, demeton, demeton-O-sulfoxide, demeton-S-methyl, demeton-S-methyl sulfone, desmedipham*, Diazinon, Dichlorobenzophenone, dichlorvos, diclobutrazole, Dicloran, Diethofencarb, difenoconazole, Diflufenican, dimethoate, *dimethomorph*, diniconazole, Diphenylamine, diuron, dodemorph, -Endosulfan, -Endosulfan, Endosulfan sulfate*, EPN, epoxiconazole, etaconazole, ethiofencarb sulfone, ethiofencarb sulfoxide, Ethion*, **ethirimol**, etofenprox, Famoxadone, fenamiphos, Fenarimol, Fenazaquin, fenbuconazole, Fenithrothion, fenoxycarb, Fenpropathrin, Fenpropidin, fenpropimorph, fenpyroximate, Fenthion, fenthion sulfoxide, Flucythrinate II, Fludioxonil*, flufenacet, Fluquinconazole, flusilazole, Flutolanil, fosthiazate, **Furalaxyl**, furathiocarb, **furmecyclox**, Heptenophos*, Hexachlorobenzene*, hexaconazole, hexythiazox, imazalil*, imidacloprid, Iprodione*, *iprovalicarb*, isoprothiolane, isoxathion, kresoxim-methyl, Lindane, linuron, Malathion, mephosfolan, Mepronil, Metalaxyl, metconazole, Methidathion, methiocarb, methiocarb sulfoxide, methomyl, methomyl-oxime, metobromuron, metoxuron, Mepanipyrim, Mevinphos, monocrotophos, monolinuron, myclobutanil, nuarimol, Ofurace*, omethoate, oxadixyl, oxamyl-oxime, oxydemeton-methyl, paclobutrazole, Parathion, Parathion-methyl*, penconazole, pencycuron, *cis*-Permethrin*, *trans*-Permethrin, phenmedipham, *o*-Phenylphenol, Phosalone*, Phosmet, phosphamidon, *Phthalimide*, picoxystrobin, Piperonyl butoxide, pirimicarb, pirimicarb-desmethyl, Pirimifos-methyl, prochloraz, Procymidone, profenofos, Prometryn, **Propargite**, Propham, propiconazole, propoxur, Propyzamide, Prothiofos, Pyrazophos*, pyridaben, pyridaphenthion, pyrifenox, pyrimethanil, Pyriproxyfen, Quinalphos*, Quinoxyfen, **sethoxydim**, spinosad, spiroxamine I, spiroxamine II, tebuconazole, tebufenozide, Tebufenpyrad, tetraconazole, **Tetradifon**, *Tetrahydrophthalimide*, thiabendazole, thiacloprid, thiamethoxam, *thiodicarb*, thiofanox, thiofanox sulfone, thiofanox sulfoxide, **thiometon**, thiometon sulfone, thiometon sulfoxide, **thiophanate-methyl**, Tolclofos-methyl, triadimefon, *triadimenol*, Triazophos*, trichlorfon*, tricyclazole, tridemorph, trifloxystrobin, triflumizole, vamidothion, vamidothion sulfone, vamidothion sulfoxide, Vinclozolin

Pesticides with 90–110% recovery (and 10 < RSD 20%): Chlorpropham, -Cyhalothrin, dicrotophos, Diphenyl, Fenpiclonil, Esfenvalerate, Etridiazole (26% RSD), Flucythrinate I, Fluvalinate, Mecarbam, oxamyl

Pesticides with 80–89% recovery: *acephate*, **cycloxydim**, **dimethomorph**, fenhexamid, Fenvalerate, **iprovalicarb**, methamidophos, *pymetrozine*, **thiodicarb**, *thiometon*

Pesticides with 70–79% recovery: **cymoxanil**, Cypermethrin, **dichlofluanid**, **florasulam**, *Furalaxyl*, *Propargite,* **triadimenol**

Pesticides with 50–69% recovery: **acephate**, **Deltamethrin**, *dichlofluanid*, *ethirimol*, *florasulam*, methiocarb sulfone, *Phosmet-oxon*, *sethoxydim*, *thiophanate-methyl*

Pesticides with 20–49% recovery: asulam, Chlorothalonil, *cycloxydim*, **pymetrozine**, pyridate, *thiram*, tolylfluanid

Nondetected pesticides: Captan, daminozide, *Deltamethrin*, Dicofol, Folpet, *furmecyclox*, **Phosmet-oxon**, **thiram**

Problematic Pesticides

The remaining 12 tested analytes (those that gave <70% recoveries in both lettuce and orange) consisted of asulam, captan, chlorothalonil, daminozide, deltamethrin, dicofol, folpet, methiocarb sulfone, phosmet-oxon, pyridate, thiram, and tolylfluanid. In reality, the actual recoveries of those pesticides that were detected by GC/MS only may have been different than the determined (or undetermined) amount because it is difficult to isolate analytical problems from sample preparation issues for these pesticides. For example, captan, folpet, dicofol, phosmet-oxon, deltamethrin, and chlorothalonil gave very inconsistent and unreliable LVI/GC/MS results. As discussed previously, several other pesticides (e.g., conazoles and certain organophosphates) also gave untrustworthy and/or inconsistent results by GC/MS but, fortunately, they were also determined by LC/MS/MS, which showed that they were recovered 100% by the QuEChERS method. Unfortunately, captan, chlorothalonil, dicofol, folpet, and some pyrethroids could not be analyzed sensitively enough by LC/MS/MS to provide more reliable results. Phosmet-oxon probably could have been added to the list of LC/MS/MS analytes, but it was not deemed important enough prior to seeing the results from these experiments.

Pyrethroids.—Analytical difficulties were clearly apparent for deltamethrin (and the other pyrethroids with low and/or inconsistent recoveries: cyhalothrin, -cyhalothrin, cypermethrin, fenvalerate, esfenvalerate, flucythrinate, and fluvalinate). Some pyrethroids (acrinathrin, bifenthrin, fenpropathrin, and *cis*- and *trans*-permethrin) gave 100 ± 10% recoveries, and previous experiments demonstrated complete recoveries of deltamethrin and permethrin (1). Therefore, it is likely that the problematic pyrethroids in this study were actually recovered completely too, but LVI/GC/MS led to lower quality results for them, probably due to irreversible adsorption onto the Carbofrit in the inlet, lower MS sensitivity in general for most late-eluting pyrethroids, and/or the condition of the capillary column, as described previously (22). Permethrin and bifenthrin do not possess the -cyano substituted group that is present in the other pyrethroids tested, thus the cause of the inconsistent results for some of the others

may be related to this part of the molecule. Deltamethrin has been shown to convert from one stereoisomer to another during GC injection in MeCN or acetone (22), albeit this conversion was not apparent in this study.

Chlorothalonil, dicofol, and N-trihalomethylthio fungicides.—Captan, folpet, dichlofluanid, and tolylfluanid represent *N*-trihalomethylthio fungicides that were included in the study. These pesticides, along with chlorothalonil and dicofol, are well-known problematic pesticides in multiresidue analysis (24). These pesticides easily degrade during sample preparation, during GC injection, and/or in solution (22), thus it is not surprising that <70% recoveries were obtained for these analytes. Usually, the degradation products of these pesticides are monitored by GC/MS, as was done in this study for captan (tetrahydrophthalimide), folpet (phthalimide), dichlofluanid (DMSA), tolylfluanid (DMST), and dicofol (dichlorobenzophenone). Figure 3 shows the recoveries in lettuce and orange for certain pairs of these analytes. The effect of pesticide degradation of dichlofluanid to DMSA and tolylfluanid to DMST are clear in the figure (as is thiophanate-methyl to carbendazim). The >110% recoveries of phthalimide and tetrahydrophthalimide in orange also hint that folpet and captan, respectively, also degraded, but the lack of degradation observed in lettuce is probably deceiving. In reality, the lettuce likely increased degradation of the parent fungicides in matrix-matched standards and sample extracts alike, which made the final results appear that recoveries of the degradation products were 100%. This almost surely explains the 100% recoveries for dichlorobenzophenone in both matrixes. Dicofol degrades rapidly in MeCN extracts to dichlorobenzophenone, thus generating an equal amount of the degradation product in sample extracts and calibration standards.

These problematic pesticides are relatively important for regulatory monitoring. They were chosen for further investigations and modifications of the QuEChERS method, and the results of that study are presented separately (31).

Others.—Asulam, daminozide, methiocarb sulfone, pyridate, and thiram are very polar analytes that are rarely included in multiresidue methods, except for methiocarb sulfone in the *N*-methylcarbamate LC method (17). They are usually analyzed separately in routine monitoring programs, if at all, and they were included in this study more out of curiosity than with the expectation that the method should be able to completely recover them. They can be analyzed semiquantitatively by the LC/MS/MS method, however, and their results in these experiments are generally believed to be accurate.

Asulam {methyl[(4-aminophenyl)sulfonyl]carbamate} is an herbicide with a unique structure containing phenyl, sulfone, ester, and primary and secondary amino groups; thus, it is not so surprising that it was not recovered completely in the method.

Daminozide was the only analyte to contain a carboxylic acid group, which is strongly retained on PSA sorbent during the dispersive-SPE cleanup step. This cleanup step is not compatible with such acidic pesticides, thus the lack of

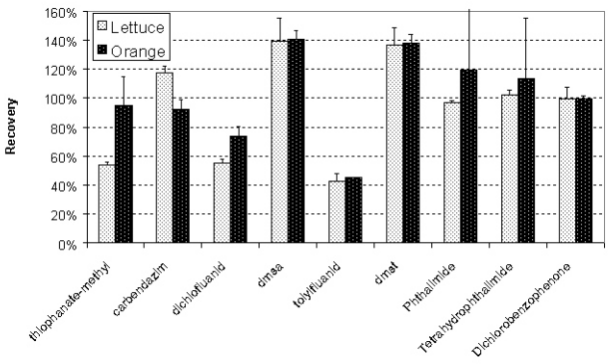

Figure 3. Recoveries of pesticides that were, or may have been, affected by degradation (carbendazim, DMSA, DMST, phthalimide, tetrahydrophthalimide, and dichlorobenzophenone are degradation products of thiophanate-methyl, dichlofluanid, tolylfluanid, folpet, captan, and dicofol, respectively).

recovery data for daminozide was expected. It was not determined what percentage of the daminozide partitioned into the MeCN phase during the extraction step relative to the effect of the cleanup step in the method.

Methiocarb sulfone was recovered >50% in both matrixes, which is still acceptable by some validation standards (32). Losses due to degradation, depending on the pH of the typical matrix, is the most likely explanation for the incomplete recoveries in this case.

Pyridate is known to be unstable (33), but the high consistency of its ca 30% recovery within and among the matrixes indicates more that it did not fully partition into the MeCN extract rather than that it partially degraded. In the situation that a physicochemical property is inducing a systematic and consistent but low recovery in a method, then a known recovery factor may be applied to provide more accurate results in real analyses.

Thiram is unstable in acidic media, like other dimethyldithiocarbamate fungicides (33). It disappeared altogether in orange and gave low recoveries from lettuce, because it was degrading more slowly at the higher pH. It also seemed to be unstable under the LC conditions used in this study.

Effect of the Matrix on Recoveries

Figures 3 and 4 show the average recoveries and standard deviations for those pesticides of special interest that mostly exhibited differences between lettuce and orange matrixes. The most likely cause of the recovery differences was pH. Oranges are quite acidic, pH ca 4, and lettuce has pH ca 6 (34), but stabilizing or degradative effects of certain matrix components could also explain the results. Either the pesticides that gave low recoveries did not completely partition into the MeCN extract from the water phase, or they degraded. Degradation can be inferred from greater variability in the results and increased response of any degradation

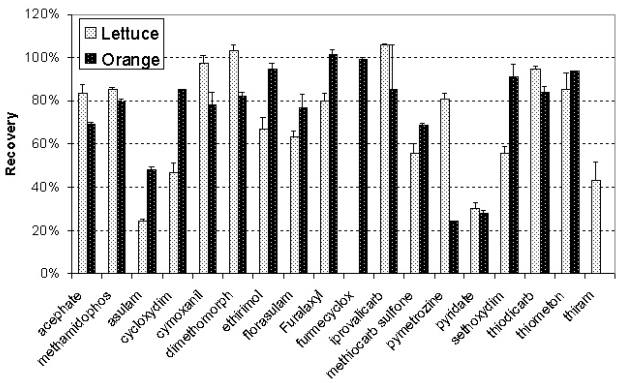

Figure 4. Recoveries of pesticides that exhibited a difference in recoveries between lettuce and orange matrixes, presumably due to pH differences.

product(s), as shown in Figure 3. Degradation products were not monitored for the pesticides listed in Figure 4, and only iprovalicarb in orange and thiometon in lettuce gave high variability. Therefore, we could not isolate whether degradation or incomplete partitioning was the cause of their lower recoveries.

As Figure 3 shows, less degradation of dichlofluanid and thiophanate-methyl occured in orange than in lettuce. As shown in Figure 4, significantly greater recoveries in orange also were observed for asulam, cycloxydim, ethirimol, florasulam, furalaxyl, furmecyclox, and sethoxydim (and methiocarb sulfone and thiometon to a smaller extent). The most dramatic difference occurred in the case of furmecyclox, which was completely recovered from orange, but completely disappeared from lettuce. This matrix-dependent recovery behavior was similarly observed during the extensive in-house validation of the miniaturized acetone extraction method at the VWA-KvW laboratory. Thiophanate-methyl, methiocarb sulfone, cycloxydim, and sethoxydim also had dramatic improvements in their recoveries in the acidic orange matrix.

On the other hand, pymetrozine gave dramatic improvements in recovery from lettuce compared to orange. Less dramatic improvements in lettuce occurred for acephate, methamidophos, cymoxanil, dimethomorph, iprovalicarb, and thiodicarb. As before (1), other basic pesticides, thiabendazole and imazilil, were unaffected by the pH differences between the 2 matrixes tested. This issue of matrix effects and pH for acid/base-sensitive pesticides is the subject of a follow-up study that is presented separately for problematic pesticides (31), and we shall not discuss these effects further in this paper.

Analysis of Proficiency Test Samples

The validation experiments demonstrated that the QuEChERS sample preparation method worked exceptionally well for nearly all pesticides commonly included in multiclass, multiresidue analysis, and many others not typically included in such methods. To further demonstrate the utility and performance of the method, we decided to analyze all of the proficiency test samples (also known as check samples) that had accumulated in the VWA-KvW laboratory since 2001. The analyst had no prior knowledge of which pesticides were present or their concentrations. All of the samples underwent thawing/freezing cycles when subsamples were taken for previous analyses (multiple times for a few samples). Therefore, homogeneity, pesticide stability, and sample integrity (including water gains or losses) were known to be possible factors that would contribute to any differences in the analytical results, but we went ahead with the experiment with the hope that these factors would not be significant. Tables 4–7 provide the results from the check sample analyses, which includes both the GC/MS and LC/MS/MS results (when applicable) from the QuEChERS method, as well as the VWA-KvW laboratory results for the same sample analyzed previously by their current method (15), the interlaboratory assigned concentrations based on the results reported for the check sample by all participating laboratories using their traditional methods, and the spiked concentration of the pesticide, if known.

In Table 4, the results are presented for 9 different samples from the FAPAS proficiency test sample program. All samples were from Series 19, which entails pesticide residue analysis of fruits and vegetables. As described previously, the LC/MS/MS results are generally more trustworthy than the GC/MS results. For example, methamidophos and omethoate concentrations determined by LC/MS/MS in melon (sample #13 from February 2001) are in closer agreement than GC/MS with the previous VWA-KvW result of the sample (using GC-flame photometric detection) and the assigned value. The same relationship occurred for myclobutanil and pyrimethanil in strawberry (#18 from December 2001), but other LC/MS/MS and GC/MS results were in very good agreement in Table 4 (penconazole in strawberry, tebuconazole in carrot, myclobutanil in apple, monocrotophos in lettuce, and diniconazole, methamidophos, and tetraconazole in sweet pepper). For convenience and as is done in common practice, matrix blanks of other commodities (lettuce and orange, in this case) were used in calibration standards rather than matrix-matched calibration specific to each matrix. This is a major drawback in the applicability of matrix-matched standards, in that not all matrixes can be matched for all sample types in a sequence of various matrixes.

Overall, the results from the FAPAS samples were in very good agreement. Chlorpyrifos in carrot (#22), deltamethrin in spinach (#17), and pirimiphos-methyl in orange (#21) gave some differences in the QuEChERS results versus the VWA-KvW, interlaboratory, and/or spiked concentrations, but such results were not out of the ordinary in interlaboratory variability and, in any event, deviations cannot be isolated to the method as the cause. Interestingly, the QuEChERS result for deltamethrin in the spinach check sample indicated complete recovery, which provided evidence that the

Table 4. Results (concentrations in ng/g) from the analysis of FAPAS (series 19) proficiency test samples analyzed in blind fashion

		QuEChERS				
Check sample	Pesticide	GC/MS[a]	LC/MS/MS[a]	VWA-KvW result[a]	Interlaboratory result[a]	Spiked concn
#13 Melon	Methamidophos	13	36	54	41 ± 9	50
	Omethoate	16	36	47	46 ± 10	50
#17 Spinach	Chlorpyrifos	52	NA	<30	40 ± 9	50
	Deltamethrin	115	NA	122	88 ± 19	120
	Metalaxyl	78	NA	80	71 ± 16	80
#18 Strawberry	Dimethomorph	NA	20	NA	NA	Incurred
	-Endosulfan I	<10	NA	NA	NA	Incurred
	-Endosulfan II	<10	NA	NA	NA	Incurred
	Endosulfan sulfate	41	NA	NA	NA	Incurred
	Malathion	107	NA	105	NA	100
	Myclobutanil	1087	894	856	712 ± 120	500
	Penconazole	78	73	76	NA	Incurred
	Pyrazophos	62	NA	46	NA	Incurred
	Pyrimethanil	287	241	249	230 ± 46	200
#21 Orange	Ethion	57	NA	54	43 ± 10	50
	Mecarbam	103	NA	126	89 ± 20	100
	Methidathion	222	NA	232	179 ± 37	200
	Pirimiphos-methyl	55	NA	92	74 ± 16	100
#22 Carrot	Chlorpyrifos	76	NA	37	52 ± 11	60
	Tebuconazole	96	109	106	83 ± 18	100
#25 Apple	Bromopropylate	107	NA	122	103 ± 23	120
	Fenvalerate	199	NA	213	149 ± 32	180
	Myclobutanil	33	31	30	32 ± 7	Incurred
	o-Phenylphenol	49	NA	54	44 ± 10	50
#26 Lettuce	Monocrotophos	88	75	82	76 ± 17	80
	Tolclofos-methyl	39	NA	46	36 ± 8	40
	Trifluralin	NA	NA	38	48 ± 11	60
#28 Orange	Chlorfenvinphos	129	NA	124	108 ± 24	150
	Parathion-methyl	65	NA	79	60 ± 13	80
#29 Sweet Pepper	Dicloran	163	NA	177	179 ± 37	200
	Diniconazole	12	13	NA	NA	Incurred
	Mecarbam	114	NA	102	90 ± 20	100
	Methamidophos	48	54	50	51 ± 11	60
	Tetraconazole	17	19	NA	NA	Incurred

[a] NA = Not analyzed and/or not applicable.

Table 5. Results (concentrations in ng/g) from the analysis of EU blind proficiency test sample #5-532 (iceberg lettuce)

	QuEChERS				
Pesticide	GC/MS[a]	LC/MS/MS[a]	VWA-KvW result[a]	Interlaboratory result[a]	Spiked concn
Acephate	71	169	147	116 ± 49	Incurred
Captan	NA	NA	NA	NA	+350[b]
-Cyhalothrin	187	NA	253	191 ± 69	+140[b]
Diazinon	111	NA	133	109 ± 22	120
Malathion	50	NA	50	65 ± 16	+100[b]
Methiocarb sulfoxide	NA	454	463	451 ± 163	500
Mevinphos	132	NA	164	167 ± 29	200
Omethoate	18	49	40	42 ± 20	52
Oxadixyl	166	141	174	153 ± 38	160
Oxydemeton-methyl	NA	181	187	166 ± 57	200
Parathion	224	NA	274	216 ± 42	260
Phosmet	230	NA	203	167 ± 43	200
Phosmet-oxon	240	NA	NA	NA	NA
Phthalimide	81	NA	NA	NA	NA
Propyzamide	369	NA	464	387 ± 64	+340[b]
Quintozene	NA	NA	100	70 ± 20	100
Tetrahydrophthalimide	412	NA	NA	NA	NA
Tolclofos-methyl	418	NA	515	431 ± 82	510

[a] NA = Not analyzed and/or not applicable.

[b] Incurred + spiked concentration.

validation results for deltamethrin could have been misleading, as described in the section on problematic pesticides.

The VWA-KvW routine method (15) involved injection of just 1 mg sample equivalent in GC/MS, whereas 5 mg was injected using LVI for the QuEChERS extracts. Thus, chlorpyrifos in spinach (#17) was not detected above the 30 ng/g reporting limit in the VWA-KvW method, but was determined at 52 ng/g by the LVI/GC/MS approach for the QuEChERS extract. Unfortunately, because not all of the same analytes were monitored in the proficiency sample testing schemes down to the 10 ng/g level, the improved sensitivity of the LVI/GC/MS method was not clearly demonstrated in the cases of endosulfans and pyrazophos in strawberry (#18) and diniconazole and tetraconazole in sweet pepper (#29). Similarly, trifluralin was not included among the 229 analytes in this study, even though it probably could have been identified easily in the QuEChERS lettuce (#26) extract by the GC/MS full-scan method.

Table 5 provides the results from a fresh (2003) EU check sample of iceberg lettuce (#5-532). Again, the results for most of the pesticides in the sample (-cyhalothrin, diazinon, malathion, methiocarb sulfoxide, mevinphos, oxadixyl, oxydemeton-methyl, parathion, phosmet, propyzamide, and tolclophos-methyl) were in good agreement. The problematic pesticides discussed above were also problematic in the traditional methods, as shown by their higher variability in the interlaboratory results. For instance, average determined acephate concentration was 116 ng/g with 42% RSD, which permits valid inclusion of both the 71 ng/g QuEChERS result from GC/MS and the 169 ng/g LC/MS/MS result (although the LC/MS/MS result is believed to be correct). A similar situation occurred for omethoate in the sample. Otherwise, the presence of multiple analytes for the same pesticides (e.g., captan/tetrahydrophthalimide and phosmet and its oxon) complicates quantitation, albeit results are still quite comparable. The proficiency test organizers had already noticed that captan was completely converted into tetrahydrophthalimide during their spiking/homogenization process, which explains the detection of the latter analyte only. In the case of phosmet, the discrepancy between the spiking level (200 ng/g) and the quantitative result of the sum of the parent and the oxon degradation product (230 + 240) can only be explained by partial (around 50%) degradation of phosmet in the sample extract and/or GC injector and a similar degree of degradation in the matrix-matched standard.

For a variety of reasons, proficiency test samples usually contain pesticides primarily detected by GC methods. With the increasing usage of modern pesticides that are not GC-amenable and greater presence of LC/MS instruments in

Table 6. Results (concentrations in ng/g) from the LC/MS/MS analysis of blind proficiency test samples supplied by the Swedish National Food Administration

Check sample	Pesticide	QuEChERS result	VWA-KvW result	Interlaboratory result	Spiked concn
LCMS1-4 Lettuce	Aldicarb sulfoxide	136	43	127 ± 55	205
	Carbendazim	41	47	42 ± 8	51
	Imazalil	40	36	36 ± 12	51
	Imidacloprid	44	40	46 ± 12	Incurred
	Methiocarb sulfone	14	31	36 ± 3	Incurred
	Methomyl	263	246	260 ± 42	308
	Oxamyl	93	70	85 ± 18	incurred
	Oxamyl oxime	59	45	64 ± 16	Incurred
	Oxydemeton-methyl	24	9	27 ± 11	31
LCMS1-68 Apple	Aldicarb	41	41	42 ± 8	51
	Aldicarb sulfone	228	227	248 ± 27	307
	Carbendazim	83	68	84 ± 23	102
	Imazalil	428	330	355 ± 153	512
	Methiocarb sulfone	69	97	101 ± 34	Incurred
	Methomyl	275	241	270 ± 30	307
	Thiabendazole	82	77	77 ± 18	102

Table 7. Results (concentrations in ng/g) from the analysis of 4 replicate EU-PT4 series proficiency test samples of orange in 4 separate containers

	QuEChERS				
Pesticide	GC/MS result[a]	LC/MS/MS result[a]	VWA-KvW result	Interlaboratory result	Spiked concn
Azinphos-methyl	ND	184 ± 6	160	210 ± 87	Incurred
Azoxystrobin	322 ± 33	321 ± 13	195	263 ± 100	340
Bromopropylate	452 ± 38	NA	430	381 ± 164	480
Carbofuran	128 ± 20	137 ± 2	141	131 ± 38	140
Chlorpyrifos	95 ± 9	NA	81	73 ± 31	Incurred
Chlorpyrifos-methyl	188 ± 16	NA	163	159 ± 64	190
Cypermethrin	431 ± 58	NA	402	386 ± 175	+350[b]
Diazinon	148 ± 10	NA	110	119 ± 40	140
Imazalil	NA	828 ± 25	1067	742 ± 266	940
Imidacloprid	NA	190 ± 4	237	NA	150
Mecarbam	187 ± 17	NA	118	126 ± 49	140
Methamidophos	NC	118 ± 3	115	92 ± 47	Incurred
Methidathion	451 ± 44	NA	334	389 ± 121	incurred
Omethoate	98 ± 22	128 ± 3	80	115 ± 62	140
Parathion	200 ± 16	NA	114	151 ± 69	200

[a] ND = Not detected; NA = not applicable; NC = not confirmed.

[b] Incurred + spiked concentration.

monitoring laboratories, the Swedish NFA provided certain laboratories with 2 check samples intended for analysis by LC/MS only. Table 6 gives the analytical results from LCMS1–4 (lettuce) and –68 (apple) samples. Very similar results were obtained for aldicarb, aldicarb sulfone, carbendazim, imazalil, imidacloprid, methomyl, and thiabendazole by all methods used. The QuEChERS results for methiocarb sulfone, which was already shown to give ca 50% recovery in lettuce and ca 70% in orange, were lower than the others by those factors in the lettuce and apple test samples, respectively. On the other hand, the VWA-KvW results for aldicarb sulfoxide, oxamyl, oxamyl-oxime, and oxydemeton-methyl in lettuce were lower than the others, in correspondence with the reported respective lower recoveries (31, 71, 60, and 40%, respectively) obtained concurrently with the standard acetone (without Na_2SO_4) extraction method. The results were not corrected for recovery by the organizer, although contemporaneously determined recoveries were required to be reported. The results for the apple sample were much the same with respect to each other for all of the other pesticides found.

Using samples from EU Proficiency Test #4 (oranges), 4 replicate analyses were performed of the sample from 4 separate containers, which allowed us to assess the repeatability of the method. Table 7 shows a comparison of the check sample results from different laboratories and methods, including the VWA-KvW and QuEChERS methods. Both the QuEChERS and original VWA-KvW results were very comparable to the assigned true concentrations and/or spiked concentrations, within the interlaboratory uncertainty intervals.

A high variability in the interlaboratory results even for nonproblematic pesticides such as methidation (31% RSD) and parathion (46% RSD) occurred in the interlaboratory analyses. Meanwhile, the QuEChERS results were no more than 4% RSD for LC/MS/MS and <11% RSD for all but cypermethrin (13%), carbofuran (16%), and omethoate (22%) for GC/MS (the latter 2 were also detected by LC/MS/MS). As before, the QuEChERS method gave very good results (typically a bit higher than the other laboratories, but closer to the added concentration in all cases except mecarbam). The QuEChERS results were likely to be accurate and, as evident from the high interlaboratory variability, the average interlaboratory results were probably lowered by laboratories that reported much lower pesticide concentrations than the others.

Side-by-Side Analysis of Routine Monitoring Samples

In an additional test of the QuEChERS method, a batch of 20 various samples from the routine monitoring program in VWA-KvW was taken for analysis by both methods. Table 8 shows the side-by-side results for the determined pesticides in the samples. As listed in the table caption, no pesticides were detected by either method in 11 of the samples. In the other 9 samples, 30 pesticide determinations above the 10 ng/g reporting limit were made by the QuEChERS approach using LC/MS/MS and LVI/GC/MS to monitor 229 pesticides, whereas 17 determinations above the average reporting limits ranging from 30–100 ng/g were made in the VWA-KvW method designed to screen for ca 350 pesticides by GC/MS and a series of selective LC and GC detection techniques (15). The 3- to 5-fold lower LOQ and chosen reporting limits of the LVI approach in GC/MS allowed detection of 9 pesticides in the samples below the corresponding reporting limits established for the routine monitoring methods, and LC/MS/MS determined 5 additional analytes (2 of which were detected by both instruments).

Figure 5 provides chromatograms and mass spectra from the LVI/GC/MS analyses that determined 20 ng/g endosulfan sulfate in eggplant and ca 3 ng/g prothiophos in plum (the latter amount was below the lowest validated level and reporting limit of 10 ng/g, hence it was not included in Table 8). The full-scan MS data aids in identification of the analytes, and independent determination of overlapping analytes with the orthogonally selective LC/MS/MS method provides an exceptional degree of qualitative and quantitative information to aid reporting decisions. Qualitatively, GC/MS and LC/MS/MS results agreed with each other in all instances (including the check samples) when concentrations exceeded the LOQ.

Quantitatively, the QuEChERS method essentially gave equivalent results (20% RSD) as the VWA-KvW method in 15 of the 17 cases in which side-by-side comparisons could be made in Table 8. In the 2 other instances (30% RSD for iprodione in grape and 68% RSD for folpet in peach), the QuEChERS result was much greater. The QuEChERS extracts were analyzed sooner after extraction than the VWA-KvW extracts, and folpet and iprodione had probably partially degraded by the time of the VWA-KvW analysis. The QuEChERS method yielded a concentration ca 30% lower than the VWA-KvW method in 7 of the 17 cases, but 5 of those were for imazalil and were likely to be a simple case of bias in the use of different calibration standards. The substantial amount of data shown in Tables 4–7 for check samples, including imazalil and several incurred pesticides, did not indicate that the QuEChERS method gave lower results.

The only curious quantitatively dissimilar result in Table 8 was for carbaryl in an orange sample. The LVI/GC/MS result from the QuEChERS method was >12 times higher than the LC methods used for the same extract and for the VWA-KvW extract. This was probably due to degradation of carbaryl in the matrix-matched GC calibration standards. As mentioned previously for carbamates and other less stable analytes, the LC/MS/MS result should be given credence over the GC/MS result.

The analyses of the many check samples and routine samples demonstrated that the QuEChERS sample preparation method was useful for several commodities other than orange and lettuce, and it could be used to provide results equivalent to the VWA-KvW method or better results (methiocarb sulfone being the only exception) than many other validated methods in current use. The inclusion of the fatty matrix, avocado, among the samples was an additional test of the method. Only prochloraz was detected in the sample, but its recovery and the recoveries of other pesticides

Table 8. Comparison of the analytical results (concentrations in ng/g) obtained using different extraction methods for real samples (no pesticides were found in apricot, French beans, nectarine, plum, pineapple, orange, cucumber, green cabbage, potato, and 2 cherry samples)

		QuEChERS		
Sample	Pesticide(s)	GC/MS	LC/MS/MS	VWA-KvW method
Avocado	Prochloraz	NA[a]	20	<LOQ
Eggplant	Endosulfan sulfate	20	NA	<LOQ
Orange	Chlorpyrifos	80	NA	90
	Imazalil	NA	1400	1700
Lemon	Imazalil	NA	1400	2000
	Prochloraz	NA	410	580
	Thiabendazole	NA	260	190
Orange	Carbaryl	120	<10	<10
	Imazalil	NA	540	620
	Thiabendazole	NA	680	1000
Clementine	Imazalil	NA	200	300
	Methidathion	20	NA	<LOQ
	Tetradifon	20	NA	<LOQ
	Thiabendazole	NA	680	1000
Orange	Carbendazim	NA	100	110
	Imazalil	NA	200	300
	Methidathion	790	NA	680
Grape	Acephate	20	<10	<LOQ
	Cyproconazole	40	40	<LOQ
	Deltamethrin	50	NA	<LOQ
	Dimethomorph	NA	240	340
	Iprodione	430	NA	230
	Famoxadone	820	NA	1000
	Pyridaben	10	<10	<LOQ
	Tebuconazole	70	60	<LOQ
Peach	Carbendazim	NA	100	90
	Folpet	160	NA	30
	Phosmet-oxon	30	NA	NA
	Phthalimide	60	NA	NA
	Thiophanate-methyl	NA	60	<LOQ

[a] NA = Not applicable

using the QuEChERS method is unknown for fatty matrixes. The usefulness of the method to extract pesticides from avocado, milk, eggs, and other fatty matrixes is the subject of a separate study (35).

Conclusions

Especially when coupled with concurrent LVI/GC/MS and LC/MS/MS analysis to provide low detection limits, the advantages of the QuEChERS extraction method in terms of quality of results (high recoveries, good repeatability and reproducibility, and wide analytical scope) and practical aspects (low cost, labor, waste, glassware, and space and high sample throughput) make it a powerful multiclass, multiresidue approach to pesticide analysis of foods. Another major benefit is that the same solvent is used for extraction of both apolar and polar pesticides and injection of the same extract, after cleanup and without an evaporation and/or concentration step, into LC and GC detection systems. The method has now undergone validation for 229 pesticides in

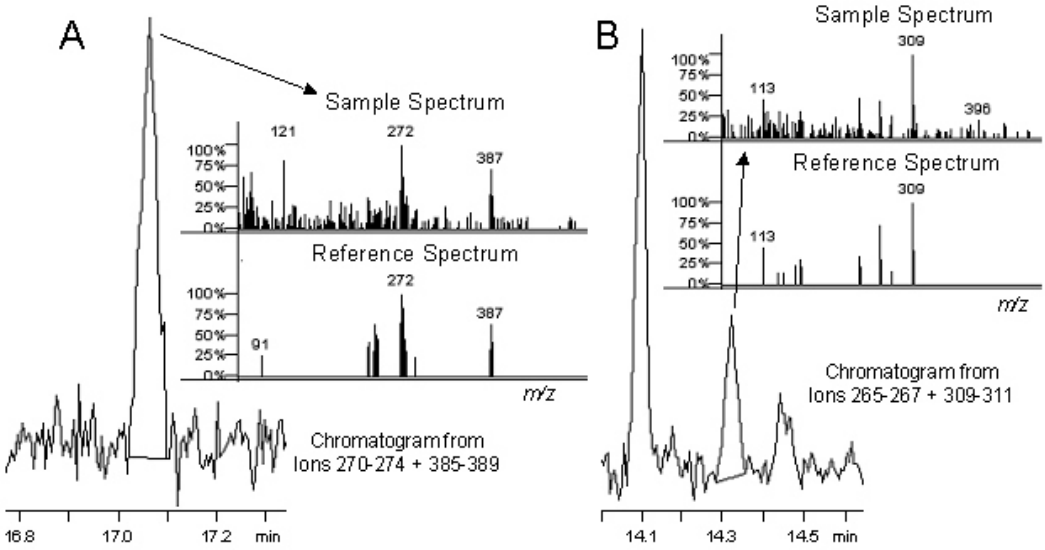

Figure 5. LVI/GC/MS detection of (A) 20 ng/g endosulfan sulfate in eggplant and (B) 3 ng/g prothiophos in plum extracted by the QuEChERS method.

2 relatively difficult, representative crops (lettuce and orange) at 3 spiking levels/crop (n = 6) as low as 10 ng/g.

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